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Isoleucine requirement of growing (25 to 45 kg) pigs[1]

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ABSTRACT: Three pig bioassays and two digestibility trials were conducted to determine the true digestible Ile requirement for maximal weight gain and minimal plasma urea nitrogen (PUN) of growing (25 to 45 kg) pigs. In Exp. 1, an Ile-deficient basal diet was developed and confirmed to be markedly deficient in Ile, yet fully efficacious when fortified with surfeit Ile. This diet contained corn, red blood cells, and soybean meal as Ile sources, and was analyzed to contain 15.5% crude protein, 0.34% Ile, and 0.95% lysine; metabolizable energy was calculated to be 3,430 kcal/kg. True digestibility of Ile in the basal diet was 89% based on digestibility trials in ileal-cannulated pigs and cecectomized roosters. The first growth trial (Exp. 2) involved six dose levels of true digestible Ile (0.38 to 0.58%), which resulted in a quadratic ($P < 0.02$) response in weight gain by growing pigs over a 21-d period. The weight-gain data were fitted to both a single-slope broken line and a quadratic fit, and when the quadratic response curve was superimposed on the broken line, the point at which the quadratic curve first intersected the plateau of the broken line occurred at 0.50% true digestible Ile. This (objective) requirement estimate was similar to that determined by taking 90% of the upper asymptote of the quadratic fitted line. In Exp. 3, a replicated 5 × 5 Latin square, five barrows (square 1) and five gilts (square 2) together with five 4-d feeding periods and five levels of true digestible Ile were utilized. A linear ($P < 0.01$) decrease in PUN occurred as Ile was incremented, with an apparent plateau occurring at 0.50% true digestible Ile. The results of these experiments suggest that the true digestible Ile requirement of grower (25 to 45 kg) pigs is 0.50% of the diet, or 1.46 g/Mcal of metabolizable energy, somewhat higher than the 1.38 g/Mcal metabolizable energy estimated by the 1998 National Research Council Subcommittee on Swine Nutrition.

Key Words: Growth, Isoleucine, Nutrient Requirements, Pigs

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Introduction

Recent research from Liu et al. (1999; 2000a,b) suggested that Ile may be among the limiting AA in today's high-lean growing and finishing pigs fed reduced-protein, all-corn, or corn-soybean meal diets. Earlier research is 20 to 50 yr old and contains problems that make interpretation difficult. Digestibility of Ile in basal diets was rarely determined, ME values were not given, and CP levels were generally very different from the levels fed in practice. Most of the early Ile work was done in pigs weighing less than 20 kg, and there was no consistency as to whether animals were fed on an ad libitum or restricted basis. These interpretive difficulties caused the NRC (1998) Committee on Swine Nutrition to base their Ile requirement estimates on a proper ratio relative to the predicted Lys requirement.

The objectives of our experiments were to develop an efficacious Ile-deficient diet based on red blood cells (**RBC**) that would show a marked growth response to supplemental Ile. Subsequently, after estimating true digestibility of Ile in the Ile-deficient basal diet, we proceeded to determine the Ile requirement for grower (25 to 45 kg) pigs based on maximal weight gain and minimal plasma urea nitrogen (**PUN**).

Materials and Methods

General Procedures

All experimental procedures were approved by the University of Illinois Committee on Laboratory Animal Care. Crossbred pigs (Line 337 males × Camborough 22 females; PIC, Franklin, KY) obtained from the University of Illinois Swine Research Center were used.

Following a 16-h period of feed deprivation, pigs in growth trials (Exp. 1 and 2) were assigned to uniform

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Table 1. Ingredients (%, as-fed basis) and chemical composition of diets for growing (25 to 45 kg) pigs

Item	Positive-control diet	Ile-deficient basal diet
Corn	72.25	85.12
Soybean meal, dehulled	23.93	3.19
Blood cells (AP301G)[a]	—	7.50
Soybean oil	1.00	1.00
Limestone	1.19	1.35
Dicalcium phosphate	1.02	1.04
Trace mineral salt[b]	0.35	0.35
Vitamin premix[c]	0.20	0.20
Tylosin premix[d]	0.02	0.02
L-Lysine·HCl	0.04	—
L-Threonine	—	0.09
DL-Methionine	—	0.15
L-Isoleucine	—	var[e]
Chemical composition		
CP, %	15.86[f]	15.50[f]
Lysine, %	0.95[g]	0.95[g]
Isoleucine, %	0.72[h]	0.34[g]
Leucine, %	1.59[h]	1.78[g]
Valine, %	0.82[h]	0.95[g]
ME, kcal/kg	3,363[h]	3,430[h]

[a]AP301G, American Protein Corp., St. Louis, MO.
[b]Supplied per kilogram of complete diet: Fe, 90 mg ($FeSO_4 \cdot H_2O$); Zn, 100 mg (ZnO); Mn, 20 mg (MnO); Cu, 8 mg ($CuSO_4 \cdot 5H_2O$); I, 0.35 mg (CaI_2); Se, 0.3 mg (Na_2SeO_3); NaCl, 3 g.
[c]Supplied per kilogram of complete diet: retinyl acetate, 2,273 μg (6,600 IU of vitamin A); cholecalciferol, 17 μg (670 IU of vitamin D_3); DL-α-tocopheryl acetate, 88 mg (88 IU of vitamin E); menadione sodium bisulfite complex, 4.7 mg; niacin, 33 mg; D-Ca-pantothenate, 24 mg; riboflavin, 8.7 mg; vitamin B_{12}, 35 μg; choline chloride, 324 mg (242 mg of choline).
[d]Tylosin premix furnished 44 mg of tylosin/kg diet.
[e]Isoleucine added to basal diet for some experiments.
[f]Analyzed by AOAC methods (AOAC, 1995).
[g]Amino acid concentrations were determined after 24-h hydrolysis in 6 *N* HCl.
[h]Calculated (NRC, 1998).

blocks based on ancestry and BW. They were then randomly allotted from within blocks to pens and treatment diets. The number of pigs per pen and pens per treatment varied with experiment. Pigs were housed in temperature-regulated barns containing pens (1.37 × 2.31 m) with partially slatted floors. Temperature was maintained at approximately 22°C, and lighting was regulated for a 16-h light/8-h dark cycle. Water and diets were provided for ad libitum consumption. At the termination of each experiment, pigs were again subjected to an overnight period of feed withdrawal, after which time pigs and feeders were weighed to allow calculation of ADG, ADFI, and gain:feed ratio.

Basal Diet: Efficacy and Isoleucine Digestibility

The Ile-deficient basal diet (Table 1) contained corn, dehulled soybean meal, and RBC (AP-301G). Following 24-h HCl hydrolysis, it was analyzed (Chung and Baker, 1992a) and found to contain 0.34% Ile, 1.78% Leu, 0.95% Val, and 0.95% Lys. Because Ile digestibility in RBC was not known, true Ile digestibility was determined in ileal-cannulated pigs and cecectomized roosters.

Red Blood Cell Digestibility in Pigs

A replicated 2 × 2 Latin square design was used to estimate true Ile digestibility in four 80-kg pigs that had been fitted with a simple T-cannula in the distal ileum as described by Wubben et al. (2001). Similar to procedures described by Mavromichalis et al. (2001), a RBC-sucrose diet and an enzymatically hydrolyzed casein (**EHC**)-sucrose diet were fed for 4 d to each pig. Both diets were fortified with vitamins and minerals to meet or exceed NRC (1998) requirements. Following each 4-d period, total ileal digesta was collected for 10 h. All diets contained 0.30% chromic oxide, and pigs were fed 2.44 kg/d, with each daily feeding divided into two equally spaced meals. The EHC diet was utilized for purposes of determining endogenous AA losses in the presence of highly digestible proteinaceous feed. Our previous work had shown that the AA in casein are nearly 100% digestible (Chung and Baker, 1992a; Mavromichalis et al., 2001). The EHC method for determining endogenous AA losses is thought to provide a more accurate prediction of endogenous losses than protein-free diet feeding (Butts et al., 1993; Donkoh et al., 1999).

Pigs were housed individually in an environmentally controlled facility with the temperature maintained at 23°C. Water was available from a nipple waterer. Digesta from each pig was kept at –20°C until analysis was performed. Samples were then thawed rapidly, after which they were lyophilized and finely ground. Diets and digesta were analyzed for Cr (Fenton and Fenton, 1979) and Ile, the latter following 24-h HCl hydrolysis.

Isoleucine Digestibility in Cecectomized Roosters

Eight cecectomized roosters were used to estimate true Ile digestibility in RBC. Four adult New Hampshire × Columbian roosters received 30 g of RBC via crop intubation, and the remaining four roosters served as fasted controls for estimation of endogenous Ile excretion. Excreta were then quantitatively collected for 48 h, after which they were frozen, freeze-dried, ground, and subjected to HCl hydrolysis and AA analysis. The RBC product (0.33% Ile) was analyzed similarly. Other procedural details of the rooster digestibility trial have been outlined by Han and Parsons (1990).

Experiment 1 (Diet Development)

The purpose of this experiment was to determine a growth-response range to Ile and to ascertain if the Ile-deficient diet fully fortified with Ile would support growth performance equal to that of a typical corn-soybean meal grower diet. Using a diet with RBC previously found to be low in Ile (Kerr et al., 2002), we formulated a basal diet that contained 0.34% Ile, 0.95% Lys, and 3,430 kcal of ME/kg (Table 1). Diets were formulated to meet or exceed NRC (1998) recommendations for all AA, with the exception of Ile. Both the corn-soybean meal positive-control diet and the Ile-fortified basal diet contained Ile at a level in excess of the NRC (1998) estimated Ile requirement (0.51%) for pigs in the weight category 20 to 50 kg (Table 1).

A total of 90 pigs were used in this experiment. Six replicates of five pigs per pen were assigned to one of three diets for a period of 21 d: 1) positive control corn-soybean meal diet, 2) negative control Ile-deficient basal diet, and 3) Ile-fortified basal diet.

Experiment 2 (Growth Assay)

The objective of this study was to determine the Ile requirement for maximal growth of 27- to 42-kg grower pigs fed graded doses of Ile. Four replicates of five pigs per pen were assigned to one of six treatments. Due to the fact that the initial basal diet validated in Exp. 1 was extremely deficient in Ile, 0.08% L-Ile was added to achieve diet 1. The test diets, therefore, included six graded levels of L-Ile. Isoleucine dose levels ranged from 0.38 (basal) to 0.58% true digestible Ile. Based on the current NRC (1998) estimates of 0.45%, for the true ileal digestible Ile requirement of pigs in the 20- to 50-kg weight category, we believed these concentrations would adequately represent both the linear and plateau regions of the growth curve.

Pigs were weighed and blocked according to ancestry and BW. After an overnight period of feed withdrawal, pigs were offered ad libitum access to their test diet for a period of 21 d. Free access to water via a single nipple waterer was available. At the termination of the experiment, pigs were subjected to overnight feed removal, after which pigs and feeders were weighed for calculation of ADG, ADFI, and feed efficiency

Experiment 3 (Plasma Urea Nitrogen Assay)

The objective of this experiment was to determine the Ile requirement of pigs based on achievement of minimal PUN. It was our belief that if the requirement obtained in the previous study was accurate, it would be validated through PUN analysis of the blood in a short-term feeding study.

Ten pigs (five barrows and five gilts) were used in a replicated 5×5 Latin square design, utilizing the lower five Ile inclusions that were used in the growth trial. The highest inclusion level of Ile from the previous experiment (0.58% true digestible Ile) was not used since it represented a level far above what appeared to be the requirement for grower pigs. Animals had ad libitum access to each test diet for a period of 4 d. At 0800 on d 5, animals were physically stimulated to awaken and consume feed. At 0900, each pig was bled via jugular venipuncture. The heparinized blood samples were stored on ice until blood collection from all pigs was complete. Samples were then centrifuged at $3{,}000 \times g$ for 15 min at 5°C, after which an aliquot of plasma from each sample was used for PUN analysis.

Statistical Analysis

Pen means data for weight gain and gain:feed (Exp. 1 and 2) and PUN (Exp. 3) were subjected to ANOVA procedures appropriate for randomized complete-block or Latin square designs (Steel and Torrie, 1980) by using the GLM procedures of SAS (SAS Inst., Inc., Cary, NC). Means were evaluated by orthogonal single df comparisons. In some cases, nonorthogonal comparisons were also made. The true digestible Ile requirement for maximal weight gain (Exp. 2) was estimated by subjecting pen means data to least squares broken-line methodology (Robbins et al., 1979). A second objective estimate from the quadratic model was determined by establishing the first point where the quadratic line intersected the plateau of the broken line. Because breakpoint plateaus of broken-line responses represent an average of animals in a population and therefore a minimal requirement estimate, the intercept of the quadratic regression curve and the plateau of the broken line were used to represent a more realistic objective estimate of the requirement. In the PUN bioassay (Exp. 3), broken-line regressions were deemed inappropriate due to poor fits. Therefore, visual best estimate

Table 2. True digestibility of isoleucine, leucine, valine, and lysine in red blood cells as determined in ileal-cannulated pigs and cecectomized roosters

	Ileal cannulated pig assay[a]		Cecectomized rooster assay[b]	
Amino acid[c]	True ileal digestibility, %	SEM	True digestibility, %	SEM
Isoleucine	97.1	0.41	96.4	2.50
Leucine	95.4	0.39	99.7	0.55
Valine	95.9	0.35	99.3	0.65
Lysine	96.3	0.35	97.0	1.08

[a]Data represent means of four pigs fitted with a simple T-cannula at the terminal ileum and fed a red blood cell diet with chromic oxide (0.30%) as a marker, in a replicated 2 × 2 Latin square design. There was a 4-d adaptation period between diet changes and a total collection for 1 d.
[b]Means of four cecectomized roosters that were crop-intubated with 30 g of RBC.
[c]Amino acid concentrations in excreta were determined in freeze-dried samples after 24-h hydrolysis in 6 *N* HCl.

plateaus (i.e., minimal PUN values) were taken to be true digestible Ile requirement estimates.

Results

True digestibility of Ile, Leu, Val, and Lys in the RBC product is shown in Table 2. True Ile digestibility values were 97.1 ± 0.41% in ileal-cannulated pigs and 96.4% in cecectomized roosters. True digestibility of other indispensable AA (Trp, Met, and cyst[e]ine not done) in RBC was high, ranging from 94.7 (Arg) to 99.8% (Tyr) in pigs and 100% (Thr) in chickens. Thus, true digestibility of AA in RBC appears to be considerably higher than that in spray-dried blood meal (NRC, 1998). Using the 97.1% true Ile digestibility value for RBC (0.33% total Ile) together with NRC (1998) true Ile digestibility values of 87% for corn (0.28% total Ile) and 89% for soybean meal (2.25% total Ile), the estimated true Ile digestibility in the Ile-deficient basal diet was 89%. Hence, the Ile-deficient pig assay diet shown in Table 1 contained 0.34% total Ile and 0.30% true digestible Ile.

In Exp. 1, pigs fed the Ile-deficient basal diet had depressed growth performance ($P < 0.01$) when compared to pigs fed the positive-control diet (Table 3). However, when the Ile-deficient basal diet was fortified with Ile to superadequacy, growth performance was restored ($P > 0.05$) to the same level as that occurring in pigs fed the positive-control diet. The results of this trial showed that the Ile-deficient diet did indeed cause a depression in growth performance, and could therefore be used in further dose-titration studies to determine the Ile requirement of grower pigs.

The results of the first Ile requirement trial (Exp. 2) are shown in Table 4. Daily weight gain and feed intake increased linearly ($P < 0.01$) to a point between 0.46 and 0.50% true digestible Ile in the diet, after which further responses were minimal. Feed efficiency results were erratic. Using single-slope, broken-line methodology (Robbins et al., 1979), a minimal breakpoint of 0.47% true digestible Ile was determined (Figure 1). The pen means data from Exp. 2 were also fitted to a quadratic regression equation:

$$Y = -2{,}306 + 11{,}289X - 10{,}480X^2 \ (r^2 = 0.61)$$

The level of true digestible Ile that maximized weight gain (i.e., upper asymptote) was calculated to be 0.54% of the diet, with 90% of this value being 0.49%. The first intercept X value of the broken line (on the plateau) and the quadratic fitted line occurred at 0.50% true digestible Ile (Figure 1). This empirical estimate of the true digestible Ile requirement is higher than the facto-

Table 3. Validation of isoleucine-deficient diet for growing pigs (Exp. 1)[a]

Diet[b]	Daily gain, g	Daily feed intake, g	Gain/feed, g/kg
1. Positive-control	689	1,457	475
2. Ile-deficient basal	209	1,047	200
3. Diet 2 + 0.30% L-Ile	644	1,369	471
Pooled SEM	33	104	22
Contrast (*P*-value)[c]			
1 vs 3	NS	NS	NS
2 vs 3	0.01	0.01	0.01

[a]Data represent means of six pens of five pigs, with an average initial BW of 25.2 kg during a 21-d feeding period.
[b]See Table 1 for composition of Ile-deficient basal diet and positive-control diet.
[c]NS indicates $P > 0.10$.

Table 4. Isoleucine levels for growing pigs (Exp. 2)[a]

Supplemental Ile, %[b]	True digestible Ile, %[c]	Daily gain, g	Daily feed intake, g	Gain:feed, g/kg
0.08	0.38	459	996	461
0.12	0.42	602	1,149	524
0.16	0.46	687	1,288	533
0.20	0.50	695	1,456	477
0.24	0.54	729	1,471	496
0.28	0.58	725	1,465	495
Pooled SEM	—	38	101	53
Contrast (*P*-value)[d]				
Linear	—	0.01	0.01	NS
Quadratic	—	0.02	0.02	NS

[a]Data represent means of four pens of five pigs with an average initial BW of 27 kg during a 21-d feeding period.
[b]See Table 1 for composition of Ile-deficient basal diet.
[c]True digestible Ile based on a digestibility coefficient of 89%.
[d]NS indicates $P > 0.10$.

rial estimate (0.45%) made by NRC (1998). Weight gain was also regressed on true digestible Ile intake, but neither broken-line nor quadratic fits were as good as those obtained when Ile concentration was used as the independent variable. Nonetheless, the first intercept X value of the broken line (on the plateau) and the quadratic fitted line occurred at a true digestible Ile intake of 727 g/d.

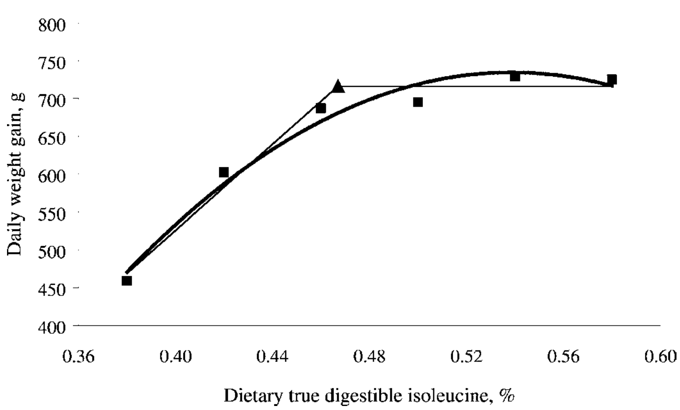

Figure 1. Fitted broken-line and quadratic plot of daily weight gain as a function of true digestible Ile in the diet (Exp. 2). Observed treatment mean values (■) are shown, but both broken-line and quadratic regression equations as well as fits (r^2) were based on pen mean values (n = 4 observations per treatment mean). The minimal true digestible Ile requirement determined by broken-line analysis using least squares methodology was 0.47% (Y plateau = 716; slope below breakpoint = 2,853; $r^2 = 0.61$). The pen means data from Table 4 were also fitted to a quadratic regression equation: $Y = -2{,}306 + 11{,}289X - 10{,}480X^2$ ($r^2 = 0.61$). The true digestible Ile level that maximized weight gain (i.e., upper asymptote) was calculated to be 0.54% of the diet, with 90% of this value being 0.49%. The first intercept X value of the broken-line (on the plateau) and the quadratic fitted line occurred at 0.50% true digestible Ile.

Plasma urea nitrogen values for pigs in Exp. 3 are shown in Table 5. Gilts had lower ($P < 0.01$) PUN values than barrows, as expected. A linear ($P < 0.01$) decrease in PUN concentration occurred as pigs consumed graded levels of Ile. An apparent plateau was reached at 0.50% true digestible Ile, and this agrees well with the Ile requirement for maximal growth determined in the growth trial. Data for barrows and gilts, both individual and combined PUN values, were fitted to quadratic regression equations to determine the Ile level that was compatible with the lower asymptote. The level of true digestible Ile that minimized PUN (lower asymptote) was 0.58%, 0.59%, and 0.58% in gilts, barrows, and gilts and barrows combined, respectively; 90% of these values were calculated to be 0.52% in gilts, 0.53% in barrows, and 0.52% in gilts and barrows combined. The quadratic fits (r^2), however, were only 0.48 for gilts, 0.26 for barrows, and 0.32 for the combined sex data. Thus, we believe the observational interpretation of 0.50% true digestible Ile is best for a confirming requirement estimate. Indeed, PUN values at the 0.54% true digestible Ile level were not lower ($P > 0.10$) than those at the 0.50% dose level for either barrows or gilts.

Discussion

Interpretation of previous Ile research is a formidable task because the primary thrust of this research occurred 20 to 50 yr ago. Today's pigs are much leaner and often have a lower appetite than pigs produced 30 yr ago. Isoleucine digestibility was rarely determined in previous studies, as illustrated by the work of Brinegar et al. (1950), Mitchell et al. (1968), Bravo et al. (1970), Pick and Meade (1970), Henry et al. (1976), and Taylor et al. (1985). Also, the majority of research was conducted with pigs weighing less than 10 kg. These factors, together with the often poor performance of pigs fed the experimental diets used, make the applicability of earlier requirement estimates questionable.

Table 5. Plasma urea nitrogen (PUN) concentration in growing pigs fed graded levels of Ile (Exp. 3)

Supplemental Ile, %[a]	True digestible Ile, %[b]	PUN in barrows, mg/dL[c]	PUN in gilts, mg/dL[d]	PUN in both sexes, mg/dL[e]
0.08	0.38	12.34	11.88	12.11
0.12	0.42	11.48	9.94	10.71
0.16	0.46	10.94	9.16	10.05
0.20	0.50	8.86	8.12	8.49
0.24	0.54	9.80	7.60	8.70
Pooled SEM	—	0.89	0.63	0.52
Contrast (*P*-value)[f]				
Linear	—	0.03	0.01	0.01
Quadratic	—	NS	NS	NS

[a]See Table 1 for composition of Ile-deficient basal diet.
[b]True digestible Ile based on a digestibility coefficient of 89%.
[c]Data represent five barrows in a 5 × 5 Latin square design; average initial BW was 26.4 kg.
[d]Data represent five gilts in a 5 × 5 Latin square design; average initial BW was 25.5 kg.
[e]Data represent 10 pigs in a replicated 5 × 5 Latin square design, with one square comprised of barrows and one square comprised of gilts.
[f]NS indicates $P > 0.10$.

More recent Ile requirement recommendations were estimated by NRC (1998). Due to the lack of relevant Ile requirement studies, however, they reviewed the plethora of Lys studies that had been carried out and estimated the Ile requirements based on ideal ratios of Ile:Lys (Wang and Fuller, 1989; Chung and Baker, 1992b).

There are several features of paramount importance in achieving an optimal requirement estimate for any AA. It is absolutely essential that the basal diet be validated to be efficacious and adequate in all nutrients except the one being studied, and it should support optimal growth performance when fully fortified. Although much previous research utilized Ile-deficient basal diets, very few actually validated the basal diet and showed it would elicit optimal performance when fully fortified with Ile. It is also important that the entire range of the growth curve (both linear and plateau portions) is reflected in the dose levels of the particular AA being studied.

Limit feeding was a popular feeding method in research work done 30 to 40 yr ago, and Becker et al. (1963), Bravo et al. (1970), Oestemer et al. (1973a,b), Brown et al. (1974), and Taylor et al. (1985) conducted their Ile requirement studies at suboptimal feed intakes. Moreover, previous Ile studies often involved dietary protein and ME levels that were very different from those used in practice. It is now commonly understood that CP and ME levels in a diet affect AA requirements, with the requirement increasing as either protein level (Becker et al., 1957; Baker et al., 1975) or energy level (Boomgaardt and Baker, 1973; NRC, 1998) increases.

The development of an Ile-deficient diet was essential to the success of the Ile requirement trials herein. Pigs fed our Ile-deficient diet not only showed a depression in performance compared to the positive control, but demonstrated a restoration in performance when surfeit Ile was supplemented. The pigs in Exp. 1 (Table 3) performed as hypothesized, indicating that a diet with 7.5% RBC was suitable for Ile dose-titration studies, 2) was limiting only in Ile, and 3) would support optimal performance when fully fortified with Ile.

The Ile digestibility studies resulted in values of 97.1 and 96.4% for true Ile digestibility in RBC for ileal-cannulated pigs and cecectomized roosters, respectively. Due to the lack of published AA digestibility values for blood products, the only available Ile comparison was the 88% true Ile digestibility of spray-dried blood meal (NRC, 1998). This was considerably lower than the 97% value obtained in our cannulated pig study. For this reason, we felt our pig estimate would be more defendable if we could repeat it using a cecectomized rooster assay. True Ile digestibility of RBC in the rooster assay was 96.4%, very close to the 97.1% value determined in pigs. Therefore, by using the pig true Ile digestibility value of 97.1% for RBC and well-documented values (NRC, 1998) for corn and soybean meal, and by calculating the contribution of each ingredient to the diet, we determined true digestibility of Ile in the basal diet to be 89%.

The newer method we are introducing as a means of predicting requirements uses both the plateau value from the one-slope broken line and the quadratic fit from the same data. The Y value obtained from the plateau of the broken-line can be substituted into the quadratic equation to obtain the equation $0 = aX^2 + bX + c$. One can then calculate the point where the quadratic curve first intersects the plateau of the broken line. It is important to note that this value is an objective estimate (i.e., calculated) and appears to be, in our judgment, a much better representation of the requirement for practical purposes, than the minimal broken-line estimate. In Exp. 2, this new method resulted in a requirement estimate of 0.50% true digestible Ile. This objective estimate is similar to the subjective estimation often used in research, wherein 90% of the upper asymptote is used. Other research in our laboratory has verified that the broken-line plateau and quadratic fit intercept value is generally in good agreement with the subjective requirement estimate obtained by determining 90% of the upper asymptotic value of a quadratic fitted line (Baker et al., 2002). We feel the newer method illustrated here for Ile (Figure 1) results in a more defendable requirement estimate because it is mathematically derived.

The results from the PUN study (Table 5) agreed very well with the growth data obtained in Exp. 2 (Table 4). Plasma urea nitrogen values should decrease as a limiting AA reaches the requirement, and PUN should plateau after the requirement has been met. The apparent plateau at 0.50% true digestible Ile indicates that PUN is a reliable indicator of the Ile requirement in pigs. This assay has potential for use in requirement studies because it represents short-term feeding, which in turn is more economical.

The Ile assay diet in these studies (Table 1) contained an estimated 3,430 kcal of ME/kg. Using this value, the true digestible Ile requirement calculates to be 1.46 g/Mcal of ME. Although NRC (1998) based their estimates of the Ile requirement on a diet containing 3,265 kcal/kg of ME, our estimate is still higher than their estimate of 1.38 g/Mcal of ME.

Implications

The Ile requirement of 25- to 45-kg pigs, as determined empirically in our studies, is higher than the NRC (1998) factorial estimate. Based on maximal weight gain and minimal plasma urea nitrogen values, the studies herein suggest the true digestible isoleucine requirement for growing pigs is 1.46 g/Mcal of metabolizable energy, somewhat higher than the NRC (1998) suggested value of 1.38 g/Mcal of metabolizable energy. Thus, diets containing blood products, and certain experimental diets used for AA studies, may require isoleucine fortification.

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